JC17 Rec'd PCT/PTO 12 JUL 2001

(1390 REV. 5-93) US DEPT. OF COMMERCE PATENT & TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER
110072

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO.
(if known, sec 37 C.F.R.1.5)

09/889178

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR00/00053	January 12, 2000	January 15, 1999

TITLE OF INVENTION
PSEUDOPEPTIDE, SYNTHESIS METHOD, REAGENT AND APPLICATIONS

APPLICANTS FOR DO/EO/US
Jean-Paul BRIAND, Vincent SEMETEY, David LIMAL

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.

3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.

    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ Entitlement to small entity status is hereby asserted.

16. ☐ Other items or information:

JC18 Rec'd PCT/PTO 12 JUL 2001

U.S. APPLICATION NO. (if known, see 37 C.F.R. 1.5) 09/889178	INTERNATIONAL APPLICATION NO. PCT/FR00/00053	ATTORNEY'S DOCKET NUMBER 110072

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $860.00		
International preliminary examination fee paid to USPTO (37 CFR1.482) $690.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $710.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) .....$ 100.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	18- 20 =	0	X $ 18.00	$	
Independent Claims	1- 3 =	0	X $ 80.00	$	
Multiple dependent claim(s)(if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$860.00	
Reduction by 1/2 for filing by small entity, if applicable.			-	$	
SUBTOTAL =				$860.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 month from the earliest claimed priority date (37 CFR 1.492(f)).			+	$	
TOTAL NATIONAL FEE =				$860.00	
				Amount to be refunded	$
				Charged	$

a. ☒ Check No. 120857 in the amount of $860.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Director is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 15-0461. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:
OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320

Date: July 12, 2001

NAME: William R. Berridge
REGISTRATION NUMBER: 30,024

NAME: Joel S. Armstrong
REGISTRATION NUMBER: 36,430

(1390 Rev.10-00)

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Jean-Paul BRIAND, Vincent SEMETEY, David LIMAL

Application No.: U.S. National Stage of PCT/FR00/00053

Filed: July 12, 2001 Docket No.: 110072

For: PSEUDOPEPTIDE, SYNTHESIS METHOD, REAGENT AND APPLICATIONS

PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

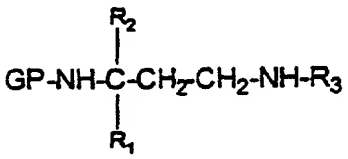
IN THE CLAIMS:

Please replace claims 3-5, 7-8, 10-12, 14-16 and 18 as follows:

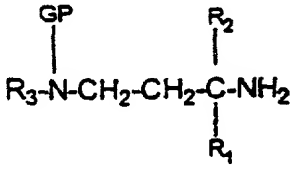
3. (Amended) The pseudopeptide as claimed in claim 1, characterized in that X represents an oxygen atom.

4. (Amended) The pseudopeptide as claimed in claim 1, characterized in that R_2 represents a hydrogen atom.

5. (Amended) A method for synthesizing a pseudopeptide as claimed in claim 1, characterized in that there is used a monoprotected diamine of general formula IIIa or IIIb



(IIIa)



(IIIb)

in which:

R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical of different, GP represents a group for protecting the amine functional group.

7. (Amended) The method as claimed in claim 5, characterized in that the carbonylating agent is chosen from N, N'-carbonyldiimidazole and triphosgene.

8. (Amended) A reagent for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, characterized in that it comprises, as reactive substance, at least one pseudopeptide as claimed in claim 1.

10. (Amended) The reagent as claimed in claim 8, characterized in that the size of the pseudopeptide is at least 12 amino acids.

11. (Amended) A kit for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, characterized in that a reagent according to claim 8, is attached to a solid support which is immunologically compatible with said reagent.

12. (Amended) A method for detecting and/or assaying biological molecules present in a sample in which the reagent as claimed in claim 8, is used to form an immune complex with said biological molecules if they are present in the sample.

14. (Amended) A method for detecting and/or assaying an antigen present in a sample by a competition technique in which said sample is brought into contact, simultaneously or in two stages, with a predetermined quantity of an antibody directed against a portion of the antigen and a predetermined quantity of a reagent as claimed in claim 8, and the presence and/or the quantity of antigen present in said sample is determined.

15. (Amended) A method for detecting and/or assaying an antibody present in a sample by a competition technique in which said sample is brought into contact simultaneously with a predetermined quantity of an antigen at least a portion of which is recognized by said

antibody and a predetermined quantity of a reagent as claimed in claim 8, and the presence and/or the quantity of anitbody present in said sample is determined.

16. (Amended) A monoclonal or polyclonal antibody which can be obtained by immunizing an animal with at least one pseudopeptide as claimed in claim 1.

18. (Amended) An active therapeutic composition, in particular an active immunotherapeutic composition, characterized in that it comprises, as active ingredient, at least one pseudopeptide as claimed in claim 1, said active ingredient being optionally in the form of a conjugate or a pharmaceutically acceptable excipient.

REMARKS

Claims 1-18 are pending. By this Preliminary Amendment, claims 3-5, 7-8, 10-12, 14-16 and 18 are amended to eliminate multiple dependencies. Prompt and favorable examination on the merits is respectfully requested.

The attached Appendix includes marked-up copies of each rewritten claim (37 C.F.R. §1.121(c)(1)(ii)).

Respectfully submitted,

William P. Berridge
Registration No. 30,024

Joel S. Armstrong
Registration No. 36,430

WPB:JSA/cmm
Attachment:
Appendix
Date: July 12, 2001

OLIFF & BERRIDGE, PLC
P.O. Box 19928
Alexandria, Virginia 22320
Telephone: (703) 836-6400

DEPOSIT ACCOUNT USE AUTHORIZATION
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Charge any fee due to our Deposit Account No. 15-0461

APPENDIX

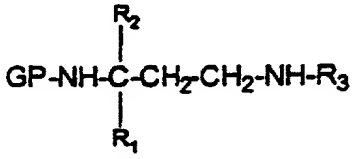
Changes to Claims:

The following are marked-up versions of the amended claims:

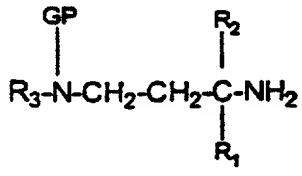
3. (Amended) The pseudopeptide as claimed in claim 1 ~~or 2~~, characterized in that X represents an oxygen atom.

4. (Amended) The pseudopeptide as claimed in ~~any one of claims 1 to 3,~~ claim 1, characterized in that R_2 represents a hydrogen atom.

5. (Amended) A method for synthesizing a pseudopeptide as claimed in ~~any one of claims 1 to 4,~~ claim 1, characterized in that there is used a monoprotected diamine of general formula IIIa or IIIb



(IIIa)



(IIIb)

in which:

R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical of different, GP represents a group for protecting the amine functional group.

7. (Amended) The method as claimed in claim 5 ~~or 6~~, characterized in that the carbonylating agent is chosen from N, N'-carbonyldiimidazole and triphosgene.

8. (Amended) A reagent for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, characterized in that it comprises, as reactive substance, at least one pseudopeptide as claimed in ~~any one of claims 1 to 4.~~ claim 1.

10. (Amended) The reagent as claimed in ~~claims 8 and 9,~~ claim 8, characterized in that the size of the pseudopeptide is at least 12 amino acids.

11. (Amended) A kit for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, characterized in that a reagent according to ~~any one of claims 8 to 10~~ claim 8, is attached to a solid support which is immunologically compatible with said reagent.

12. (Amended) A method for detecting and/or assaying biological molecules present in a sample in which the reagent as claimed in ~~any one of claims 8 to 10~~ claim 8, is used to form an immune complex with said biological molecules if they are present in the sample.

14. (Amended) A method for detecting and/or assaying an antigen present in a sample by a competition technique in which said sample is brought into contact, simultaneously or in two stages, with a predetermined quantity of an antibody directed against a portion of the antigen and a predetermined quantity of a reagent as claimed in ~~any one of claims 8 to 10,~~ claim 8, and the presence and/or the quantity of antigen present in said sample is determined.

15. (Amended) A method for detecting and/or assaying an antibody present in a sample by a competition technique in which said sample is brought into contact simultaneously with a predetermined quantity of an antigen at least a portion of which is recognized by said antibody and a predetermined quantity of a reagent as claimed in ~~one of claims 8 to 10,~~ claim 8, and the presence and/or the quantity of anitbody present in said sample is determined.

16. (Amended) A monoclonal or polyclonal antibody which can be obtained by immunizing an animal with at least one pseudopeptide as claimed in ~~any one of claims 1 to 4.~~ claim 1.

18. (Amended) An active therapeutic composition, in particular an active immunotherapeutic composition, characterized in that it comprises, as active ingredient, at least one pseudopeptide as claimed in ~~any one of claims 1 to 4,~~ claim 1, ~~an antibody as claimed in claim 16, or an anit-idiotype as claimed in claim 17,~~ said active ingredient being optionally in the form of a conjugate or a pharmaceutically acceptable excipient.

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of

Jean-Paul BRIAND et al.

Application No.: 09/889,178

Filed: July 12, 2001 Docket No.: 110072

For: PSEUDOPEPTIDE, SYNTHESIS METHOD, REAGENT AND APPLICATIONS

SUPPLEMENTAL PRELIMINARY AMENDMENT

Director of the U.S. Patent and Trademark Office
Washington, D. C. 20231

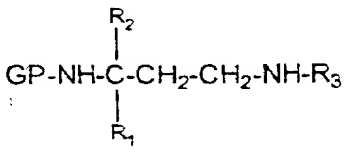
Sir:

Prior to initial examination, please amend the above-identified application as follows:

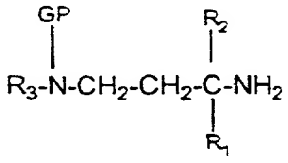
IN THE CLAIMS:

Please replace claim 5 as follows:

5. (Amended) A method for synthesizing a pseudopeptide as claimed in claim 1, characterized in that a monoprotected diamine of general formula IIIa or IIIb



(IIIa)



(IIIb)

in which: R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical or different, GP represents a group for protecting the amine functional group is coupled with an amine in the presence of a carbonylating agent.

REMARKS

Claims 1-18 are pending. By this Preliminary Amendment, claim 5 is amended. Prompt and favorable examination on the merits is respectfully requested.

The attached Appendix includes marked-up copies of each rewritten claim (37 C.F.R. §1.121(c)(1)(ii)).

Respectfully submitted,

William P. Berridge
Registration No. 30,024

Joel S. Armstrong
Registration No. 36,430

WPB:JSA/cmm

Attachment:
Appendix

Date: August 21, 2001

OLIFF & BERRIDGE, PLC
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DEPOSIT ACCOUNT USE AUTHORIZATION
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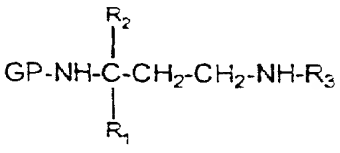
APPENDIX

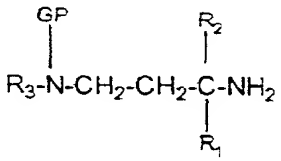
Changes to Claims:

The following is a marked-up version of the amended claim:

5. (Amended) A method for synthesizing a pseudopeptide as claimed in claim 1, characterized in that ~~there is used~~ a monoprotected diamine of general formula IIIa or IIIb



(IIIa)



(IIIb)

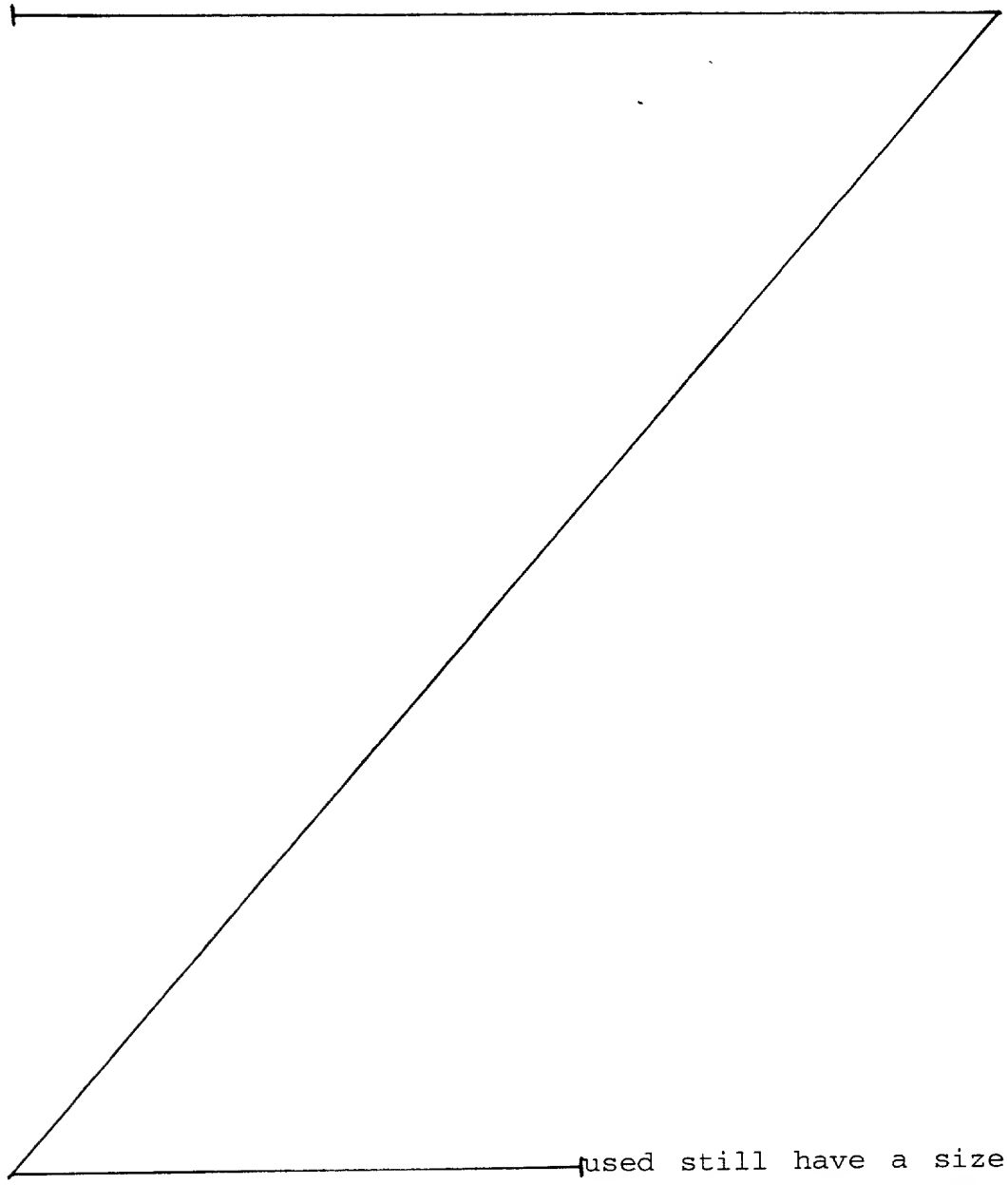
in which: R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical or different, GP represents a group for protecting the amine functional group. is coupled with an amine in the presence of a carbonylating agent.

PSEUDOPEPTIDE, SYNTHESIS METHOD, REAGENT AND APPLICATIONS

For many years, many teams have focused on synthesizing analogs of peptides or proteins which mimic the biological activities of natural peptides or proteins. There may be mentioned, by way of example, the peptide analogs obtained by replacing one or more amino acids of the L series with one or more corresponding amino acids of the D series, the peptides exhibiting a modification at the level of at least one of the peptide bonds, such as the retro, inverso, retro-inverso, carba and aza bonds.

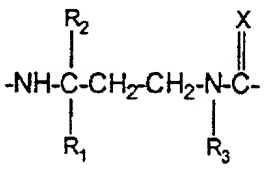
The carba bond (CH_2-CH_2) has been described as a potential mimic of the peptide bond (Mendre C. et al., European J. Pharmacol., 186, p. 213-222, 1990; Attwood et al., Bioorg. Med. Chem. Lett., 7, p. 429-432, 1997). Moreover, the partial or complete replacement of the α-carbon by a nitrogen atom on a peptide has made it possible to obtain advantageous pseudopeptides called azapeptides and azatides respectively (Gante, J., Synthesis, p. 405-413, 1989; Han H. and Janda K.D., J. Amer. Chem. Soc, 118, p. 2539-2544, 1996).

In general, these peptide analogs, called pseudopeptides, have, as a first advantage, a metabolic stability which is greater than that of natural peptides or proteins because they are not degraded by natural proteases or are degraded less rapidly. Moreover, the conformational changes induced by these chemical modifications can improve the biological properties of these pseudopeptides, see for example the decapeptide analogs which are antagonists of the hypothalmic hormones and which are described in WO-A-92/13883:

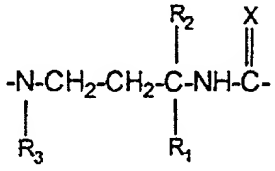


used still have a size greater than at least 12 amino acids (D. Osmanov, AIDS, 5(1), WHO1-WHO9, 1991). In another example, such as the diagnosis of Chagas' disease, the peptides used comprise a minimum of 12 amino acids (WO-A-97/18475). In (Bradshaw C.G. et col., J. Med. Chem., 37, 1991-1995, 1994) fluorescent probes which are analogs of the heptapeptide antagonist of NK_2 were obtained by substitution of an amino acid and coupling with a fluorophore.

It is the object of the present invention to describe a novel family of pseudopeptides comprising a novel carbaza unit significantly modifying the peptide backbone and whose use in the context of peptide synthesis is easy both in solid phase and in liquid phase, and this even for peptides of a large size and in particular greater than 6 amino acids. This novel family of pseudopeptides can be used in the diagnostic field to provide in vitro methods for the diagnosis of pathology conditions associated with the presence of endogenous or exogenous proteins in an individual, or in the therapeutic field, and in particular immunotherapy or vaccination. These pseudopeptides have a size of at least 6 amino acids comprising at least one unit chosen from the B units of general formula I and/or II defined below:



(I)



(II)

in which:

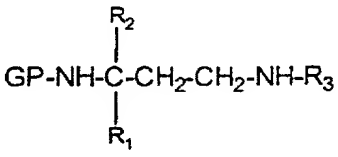
and the non-natural amino acids. Examples of these non-natural amino acids are given in the Novabiochem catalog (Catalog & Peptide synthesis Handbook; 1999; CH-4448, Läufelfinfgen, Switzerland) or the Néosystem catalog (Catalog 1997/1998; 67100 Strasbourg, France).

The expression amino acids side chain is understood to mean all the side chains of the amino acids as defined above. In the case of proline, it is understood that the side chain R_1 or R_2 in the formula of the B unit cyclizes so as to bond to the nitrogen in the alpha position. Likewise, R_1 and R_2 may bind covalently.

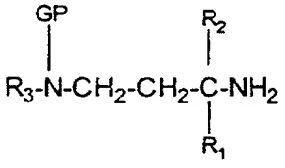
Preferably, the pseudopeptide comprises at least 9 amino acids. Advantageously, in the case of diagnosis, the pseudopeptide comprises at least 12 amino acids. The B unit as defined represents 2 amino acids since the linear backbone of said B unit possesses a structure with 6 atoms.

Preferably, the NH functional group of formula I and the NR_3 functional group of formula II are linked to a group CX, and/or the CX functional group of formulae I and II are linked to a group NH or NR_3, said groups CX, NH and NR_3 belonging to a peptide or pseudopeptide unit.

The invention also relates to a method for synthesizing the pseudopeptide containing at least one B unit. For that, the molecule(s) required are monoprotected diamines having the following structure IIIa or IIIb:



IIIa



IIIb

where

GP represents any group for protecting the amine functional group, such as for example those described in T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Synthesis, 2nd edition, John Wiley and Sons, New York, 1991; preferably those commonly used in peptide synthesis, namely:
Boc (tert-butyloxycarbonyl),
Fmoc (9-fluorenylmethyleneoxycarbonyl),
Cbz (carboxybenzyl), or
Alloc (allyloxycarbonyl), and
R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical or different.

This molecule is then coupled to an amine via a carbonylating agent. By way of example, there may be mentioned N,N'-carbonyldiimidazole (CDI) (Zhang, X.; Rodrigues, J.; Evans, L.; Hinckle, B.; Ballantyne, L.; Pena, M. J. Org. Chem. 62, 6420-6423, 1997), p-nitrophenyl carbamate (Hutchins, S.M. & Chapman K.T. Tet Lett. 36, 2583-2586, 1995), 2,4-dinitrophenyl carbonate (Quibell, M.; Turnell, W.G.; Johnson, T. J. Chem. Soc. Perkin Trans. I 2843-2849), N,N'-disuccunimidyl carbonate (DSC) (Takeda, K.; Akagi, Y.; Saiki, A.; Tsukahara, T.; Ogura, H. Tet. Lett. 1983, 24, 4569-4572) and more particularly triphosgene (Majer, P. & Randad, R.S. J. Org. Chem. 59, 1937-1938, 1994). This reaction may be carried out on a solid support or in homogeneous phase.

Using this coupling technique, the B unit may be introduced at any position of the pseudopeptide and it is easy to prepare a pseudopeptide comprising several B units corresponding to the formulae I and/or II. The pseudopeptide may comprise exclusively a succession of B units corresponding to the formulae I and/or II.

The pseudopeptide according to the invention may be modified after or during the synthesis, for example by coupling with tracers, ligands or anti-ligands, proteins, vitamins, by phosphorylation, sulfation, glycosylation or hydroxylation. The pairs biotin/streptavidin, lectin/sugar, hapten/antibody, chelator/chelated molecules, hormone/receptor, polynucleotide/complementary polynucleotide are examples of ligand/anti-ligand pairs.

An example of a strategy for modifying a peptide with biotin is given in Limal, D.; Briand, J.P.; Dalbon, P.; Jolivet, M., 1998, J. Peptide Res. 52, 121-129.

The structure of the pseudopeptide may undergo modifications such as intrapeptide or interpeptide bonds. As examples for the formation of an intrapeptide bond, the creation of disulfide bridges between various cysteine side chains or the formation of lactams between two side chains or between the two C-terminal and N-terminal ends may be envisaged. The interpeptide bonds may lead to the formation of peptide multimers crosslinked or otherwise by the use of bifunctional coupling reagents.

Examples of a coupling strategy for the modification of the pseudopeptide are given in Chemistry of protein conjugation and cross-linking, Wong S.S., CRC Press, Boca Raton, 1991 or in Bioconjugate techniques, Hermanson G.T., Academic Press, San Diego, 1996. The pseudopeptides according to the invention may be linear cyclic or branched.

The synthesis of the pseudopeptide may be carried out on a solid support by conventional recurring techniques, by chemical ligation techniques (W. Lu et al., FEBS Letters, 429, p. 31-35, 1998 or J.A. Camarero et al., J. Peptide Res., 51, p. 303-316, 1998) or by fragment condensing techniques (Chemical approaches to

the synthesis of peptides and proteins, Lloyd-Williams P., Albericio F., Giralt E., CRC Press, Boca Raton, 1997) or by combining these various techniques.

Another subject of the invention is a reagent for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, said reagent comprising, in addition, a pseudopeptide of the invention as reactive substance. The pseudopeptide is advantageously labeled with a tracer or biotin. Preferably, the size of the pseudopeptide is at least 12 amino acids.

The pathological conditions may all relate to animal or human pathologies and in particular human pathologies, and in particular pathological conditions of viral or parasitic origin, the field of cancer, autoimmune diseases or neurodegenerative diseases.

The detection of pathological conditions may be carried out in a direct or indirect manner. The term direct is understood to mean the detection of this pathological condition in a biological sample obtained from the human or animal organism such as, for example, blood, urine, sputum or a smear. The term indirect is understood to mean the detection of proteins in samples such as, for example, water, air, food, pharmaceutical products, cosmetics which may come into contact with said human or animal organism to cause a pathological condition.

The subject of the invention is a kit for detecting pathological conditions associated with the presence of endogenous or exogenous proteins comprising the reagent described above, attached to a solid support which is immunologically compatible with said reagent.

The term "solid support" as used here includes all the materials on which a biological molecule may be

immobilized for use in diagnostic tests and in separation processes. Natural or synthetic materials, chemically modified or otherwise, may be used as a solid support, in particular polysaccharides such as cellulose-based materials, for example paper, cellulose derivatives such as cellulose acetate and nitrocellulose, dextran; polymers such as polyvinyl chlorides, polyethylenes, polystyrenes, polyacrylates, polyamides or copolymers based on monomers of the styrene type, esters of unsaturated carboxylic acids, vinylidene chloride, dienes or compounds having nitrile functional groups (such as acrylonitrile); copolymers vinyl chloride/propylene, vinyl chloride/vinyl acetate; natural fibers such as cotton and synthetic fibers such as nylon; inorganic materials such as silica, quartz glass ceramics; latexes, that is to say aqueous colloidal dispersions of any polymer which is insoluble in water; magnetic particles; metallic derivatives, and the like.

The solid support may be in particular in the form of a microtiter plate, a sheet, a cone, a tube, beads, particles and the like.

The attachment of the reagent may be carried out in a direct or indirect manner.

In the direct manner, two approaches are possible: either by adsorption of the reagent onto the solid support, or by covalent bonding. In one variant, the pseudopeptide of the reagent may be coupled to a polypeptide, a protein or a nucleic acid fragment in order to enhance the attachment onto the solid phase.

In the indirect manner, it is possible to attach beforehand (by covalent bonding or adsorption) an anti-reagent capable of interacting with the reagent so as to immobilize the whole onto the solid support. By way of example, streptavidin, adsorbed onto the solid

support, can allow the attachment of a pseudopeptide carrying a biotin, or an antibody (monoclonal, polyclonal or an antibody fragment) directed against the B unit of the invention can allow this same attachment of the pseudopeptide.

The invention relates, in addition, to a method for detecting and/or assaying biological molecules, and in particular antibodies, present in a sample in which the reagent according to the invention is used to form an immune complex with said biological molecules if they are present in the sample.

The invention relates in particular to a method for detecting and/or assaying antibodies in a sample, comprising the steps consisting in bringing said sample into contact with a reagent of the invention under conditions allowing an immunological reaction, and then in detecting and/or assaying the immune complex which may be formed.

In a particular mode, the reagent of the invention is attached to the solid phase and the immune complex is detected with the aid of a second antibody labeled with a tracer.

In another particular mode, the immune complex between the labeled reagent and the biological molecule is formed in the homogeneous phase and its presence is detected by a physicochemical modification of the tracer linked to the formation of the immune complex.

By way of example, this second antibody is a monoclonal or polyclonal antibody or an Fab-type fragment, directed for example against human antibodies in the case of a human biological sample.

The term tracer is intended to mean an entity capable of generating a detectable signal.

The tracer may be chosen in particular from:

- enzymes which produce a signal which can be detected for example by colorimetry, fluorescence, luminescence, such as horseradish peroxidase, alkaline phosphatase, β-galactosidase, glucose-6-phosphate dehydrogenase,

- chromophores such as fluorescent compounds, luminescent compounds or colorants,

- groups with an electron density which can be detected by electron microscopy or by their electrical properties such as conductivity, amperometry, voltametry, impedance measurements,

- groups which can be detected by optical methods such as diffraction, surface plasmon resonance, contact angle variation or physical methods such as atomic force spectroscopy or tunnel effect.

The labeling with a tracer may be carried out either in a direct or indirect manner.

The expression direct labeling is understood to mean the covalent attachment of the tracer. The expression indirect labeling is understood to mean the noncovalent attachment of the tracer, in particular by ligand/anti-ligand interactions.

The invention also relates to a method for detecting and/or assaying an antigen present in a sample by a competition technique in which said sample is brought into contact, simultaneously or in two stages, with a predetermined quantity of an antibody directed against a portion of the antigen and a predetermined quantity of a reagent of the invention, and the presence and/or

the quantity of antigen present in said sample is determined.

In a particular mode, it is the antibody which is attached to the solid phase and the reagent of the invention is labeled with a tracer.

The invention also relates to a method for detecting and/or assaying an antibody present in a sample by a competition technique in which said sample is brought into contact simultaneously with a predetermined quantity of an antigen at least a portion of which is recognized by said antibody and a predetermined quantity of a reagent of the invention, and the presence and/or the quantity of antibody present in said sample is determined.

In a particular embodiment, the antigen is attached to the solid phase and the reagent of the invention is labeled with a tracer. In another variant, the reagent is attached to the solid phase and the antigen is labeled with a tracer.

The pseudopeptides of the invention are in addition of interest in the production of vaccines. It is now established that peptide analogs have a capacity to stimulate the T lymphocytes (P. Aichele et al., 1995. T cell priming versus T cell tolerance induced by synthetic peptide. J. Exp. Med. 182:261, S. Tourdot et al., 1997. Chimeric peptides: a new approach to enhancing the immunogenicity of peptides with low MHC class I affinity: application in antiviral vaccination. J. Immunol. 159:2391).

Thus, the subject of the invention is also the antibodies directed against the pseudopeptides according to the invention which may be monoclonal or polyclonal. Said antibodies are capable of being obtained by immunizing an animal with at least one

pseudopeptide according to the invention. The antibodies according to the invention are more particularly characterized in that they are capable of forming a complex with pseudopeptides and/or the parent proteins or peptides corresponding to the latter.

The expression parent protein is understood to mean a natural protein and the expression parent peptide is understood to mean

either a peptide which exists as such in the natural state, in particular in a higher organism, and in particular the human body,

or a peptide derived from a protein as it exists in the natural state in the abovementioned organisms, in particular by fragmentation of said protein or by peptide synthesis,

or a peptide of immunological interest which is obtained by peptide synthesis,

or a peptide derived from a protein as it exists in the natural state but whose immunological activity has been modified, preserved or optimized by replacing certain amino acids, such as for example following a screening of a library of analogous peptides obtained by peptide synthesis.

The anti-pseudopeptide antibodies of the invention recognize the parent peptide or the parent protein with an affinity at least equal to that exhibited by the anti-parent peptide or anti-parent protein antibodies toward the parent peptide or the parent protein. The affinity constant at equilibrium Ka of the complexes is a means of measuring the affinity.

The invention also relates to the anti-idiotypes which can be obtained by immunizing an animal with said antibodies as defined above.

During recent studies, some authors, including those of the present invention (J.P. Briand et al., 1997. A retro-inverso peptide corresponding to the GH loop of foot-and-mouth disease virus elicits high levels of long-lasting protective neutralizing antibodies. Proc. Natl. Sci. USA 94:12545; C. Stemmer et al., 1999. Protection against lymphocytic choriomeningitis virus infection induced by a reduced peptide bond analogue of the H-2D^b-restricted CD8(+) T cel epitope GP33. J. Biol. Chem. 274:5550), have shown that peptide analogs may advantageously replace natural peptides, in therapy. By way of example, it has been observed that the modifications of the peptide backbone can considerably influence the interactions of the MHC complex/peptide with the receptor for the T lymphocytes (C. Stemmer et al., 1999. Protection against lymphocytic choriomeningitis virus infection induced by a reduced peptide bond analogue of the H-2D^b-restricted CD8(+) T cel epitope GP33. J. Biol. Chem. 274:5550; M. Ostankovitch et al., 1998. A partially modified retro-inverso pseudopeptide modulates the cytokine profile of CTL specific for an influenza virus epitope. J. Immunol. 161:200; S. Calbo et al., 1999. Role of peptide backbone in T cel recognition. J. Immunol. 162:4657).

Another application of the pseudopeptides according to the invention is an active therapeutic composition and in particular an active immunotherapeutic composition, preferably a vaccine composition comprising, as active ingredient, a pseudopeptide having a half-life greater than that of the natural proteins or that of the synthetic peptides derived or otherwise from these natural proteins (these natural proteins, or these peptides derived or otherwise from the latter being

designated by the expression parent proteins or peptides) of which they are analogs, said active ingredient being optionally in the form of a conjugate or a pharmaceutically acceptable excipient.

The abovementioned pathological conditions which may be treated in the context of the present invention are mainly either diseases of viral, bacterial or parasitic origin, when they are associated with the presence of the microorganism itself, or autoimmune diseases when they are associated with the presence of endogenous proteins or peptides disrupting the normal physiological function of an organism when the latter directly play a role of antibody or induce the formation of antibodies recognizing and altering particular sites of the organism such as, for example, by forming deposits of antibody/antigen complexes or by causing inflammatory states. The abovementioned pathological conditions may also be neurodegenerative diseases when they are associated with the presence, in the organism, of exogenous proteins having the effect of causing neurological lesions. The pseudopeptides used for the preparation of the pharmaceutical compositions or vaccines are advantageously pseudopeptides whose backbone consists solely of a succession of B units of general formula I and/or II.

The invention relates more particularly to the use of a pseudopeptide as defined above, for the preparation of a vaccine in the context of the prevention of pathological conditions associated with the presence, in the body of an individual, of one or more exogenous or endogenous proteins which may be recognized by antibodies directed against the pseudopeptides or directed against the anti-idiotypes according to the invention.

The invention also relates to any pharmaceutical composition comprising at least one pseudopeptide as

defined above or at least one abovementioned anti-idiotype, combined with a protein or nonprotein carrier molecule which may induce *in vivo* the production of antibodies neutralizing said exogenous or endogenous proteins responsible for the pathological condition, or induce *in vivo* a cytotoxic immune cell response. The invention relates, in addition, to any pharmaceutical composition comprising at least one antibody defined above.

As regards the use of the pseudopeptides in the context of medicaments intended for the treatment of autoimmune diseases, it should be recalled that the pathogenesis of numerous autoimmune diseases involves the presentation of autoantigens (attached to MHC molecules) to the receptor for autoreactive T cells which have somehow escaped the self-tolerance process. Accordingly, the development of novel strategies for modulating the autoreactive T cells response could lead to therapeutic approaches capable of treating certain autoimmune diseases.

Certain autoimmune diseases are associated with specific MHC I or II alleles. Thus, the use of blocking peptides capable of interacting with a given MHC molecule (for example an MHC molecule class II associated with a particular autoimmune disease) but which cannot activate the pathogenic T cell response is promising. However, the degradation of the peptides in the biological media makes their use difficult. In this case, the pseuopeptides, by virtue of their stability, could be very advantageous.

The following examples make it possible to illustrate a few advantages of the invention without, however, limiting the scope thereof. They refer to the accompanying drawing in which:

- Figure 1 illustrates the synthesis of a diamine responsible for mimicking the dipeptide sequence (Ala-Val) once introduced into a peptide; this synthesis was carried out according to the two strategies commonly used in peptide synthesis (Boc and Fmoc) to demonstrate the general features of the route proposed;

- Figure 2 illustrates the introduction, onto a solid support, of the amine monoprotected via carbonylation, leading to the isocyanate.

Example 1: Synthesis of a protected diamine for the introduction of the carbaza unit B into a pseudopeptide.

The route of synthesis used to obtain this amino acid derivative is the following.

A natural or non-natural N-protected amino acid is first of all converted by the action of diazomethane in order to obtain the corresponding N-protected diazoketone (**1**, Figure 1). The N,O-dimethylhydroxamate of the N-protected β-amino acid **2** is then obtained, by direct Wolff rearrangement in the presence of N,O-dimethylhydroxylamine, according to the method described by Limal et al. (Limal, D.; Quesnel, A.; Briand, J.P. Tet. Lett. 39, 4239-4242, 1998) (2, Figure 1). This step may be carried out in a more conventional manner, passing via the N-protected β-amino acid. The reduction of this molecule into an aldehyde is carried out by the method described by Fehrentz and Castro (Fehrentz, J.A. & Castro, B. Synthesis 676-678, 1982). A reductive amination between the aldehyde obtained and a protected primary amine leads to the N-protected diamine **3**. The protection of the primary amine will be orthogonal to the first protection of the amine acid so as to be able to selectively remove one of the two. By way of example,

in the case of an amino acid which is N-protected by a Boc group, the amine to be introduced will be protected by an allyl or benzyl group, while in the case of an amino acid which is N-protected by an Fmoc group, the amine to be introduced will be protected by an allyl group. The deprotection of this group then makes it possible to obtain the monoprotected diamine **4a** or **4b**.

By way of example, figure 1 illustrates the synthesis of a diamine which mimics the dipeptide sequence (Ala-Val) once introduced into a peptide. This synthesis was carried out according to the two strategies commonly used in peptide synthesis (Boc and Fmoc) to demonstrate the general nature of the route proposed. The reaction yields are indicated for each step.

The procedure for this synthesis is described below according to the various steps:

(1) During reaction (1), there are reacted with 1 equivalent of commercial amino acid corresponding to alanine N-protected by the protecting group Boc (Novabiochem, reference 04-12-0002) or the protecting group Fmoc (Novabiochem, reference 04-12-1006), 1.1 equivalents of iBuOCOCl (sold by the company Aldrich, St Quentin Fallavier, France under the reference 17,798-9) and 1.1 equivalents of NMM (4-methylmorpholine, Aldrich, 40770-4), in THF (tetrahydrofuran, Aldrich, 40175-7) at an amino acid concentration of 0.1 molar at the temperature of -25°C for 1 hour. The intermediate product is filtered in order to remove the salts formed.

(2) This intermediate product then reacts (step (2)) with diazomethane CH_2N_2 (prepared from a Diazald precursor sold by the company Aldrich, reference D2,800-0 using the specific setup sold by the company Aldrich under the reference Z10,851-0) in solution in ether, at room temperature, for 2 hours. The solvents

are evaporated off with the aid of a rotary evaporator and product **1** is purified by silica chromatography with an ethyl acetate/hexane:30/70 mixture).

(3) Reaction (3) is carried out by mixing 1 equivalent of product **1** with 3 equivalents of Et_3N, 0.15 equivalent of $C_6H_5CO_2Ag$ (Aldrich, 22,727-7) in THF (0.1 molar with respect to product **1**) and then adding 1.5 equivalents of HN(OMe)Me (obtained by neutralizing with 2 equivalents of NEt_3 the acidic precursor sold by Aldrich reference D16,370-8) at a temperature of -25°C. The reaction mixture is brought to room temperature for 2 hours. After concentrating the solvents, washing with a potassium sulfate solution, drying over magnesium sulfate, evaporating the organic solvents and purifying by silica chromatography with an ethyl acetate/hexane:50/50 mixture, product **2** is isolated.

(4) Reaction (4) is carried out by reacting 3 equivalents of $LiAlH_4$ in THF (Aldrich, 21776-6) at a concentration of 0.1 molar with respect to product **2** at the temperature of -30°C for 1 hour. 50 ml of ethyl acetate are added to the reaction mixture. The excess hydride is then neutralized by adding an aqueous potassium hydrogen sulfate solution, the organic phase is successively washed with a potassium hydrogen carbonate solution and then with a saturated NaCl solution. The organic phase is dried over magnesium sulfate, filtered and evaporated to give the corresponding aldehyde.

(5) Reaction (5) is carried out by reacting 1.1 equivalents of *N*-isopropylbenzylamine (Aldrich, 13,696-4) and 1.4 equivalents of $NaBH(OAc)_3$ (Aldrich, 31,639-3) in DCE (1,2-dichloroethane, Aldrich, 31992-9) at a concentration of 0.3 molar with respect to product **2a** at room temperature for 3 hours. The reaction mixture is treated as indicated in step (4) after evaporation of the DCE.

(6) Reaction (6) is a catalytic hydrogenation carried out in methanol at 0.1 molar with respect to product **3a** in the presence of 0.1 equivalent of the reagent palladium on carbon bed (Aldrich, 20,569-9). The reaction mixture is then filtered in order to remove the catalyst and, after evaporating the solvent, product **4a** is obtained.

(7) Reaction (7) is carried out by reacting 1.1 equivalents of *N*-isopropylallylamine and 1.4 equivalents of $NaBH(OAc)_3$ in DCE at a concentration of 0.3 molar with respect to product **2** at room temperature for 3 hours. The treatment carried out to obtain product **3b** or **3c** is identical to that of step (4).

The synthesis of N-isopropylallylamine is the following.

Allyl bromide (100 mmol, Aldrich, A2,958-5) is slowly added to a stirred solution of isopropylamine (200 mmol, Aldrich 10,906-1), in 40 ml of water, at room temperature. The reaction mixture is then heated under reflux over a period of 4 hours. 10 g of sodium hydroxide (250 mmol) are added to the mixture at 10°C, and the mixture is kept stirred for 1 hour while allowing the temperature to rise to 20°C. The mixture is extracted with ether (twice 30 ml) and then the organic phase is dried over Na_2SO_4 and the solvent is evaporated off. The residue is distilled until the expected product is obtained (boiling point 79°C).

(8) Reaction (8) is carried out by reacting 0.05 equivalent of a mixture $Pd(dba)_2$ (bis(dibenzylideneacetone)palladium(0), sold under the reference 8764 by the company Lancaster, Strasbourg, France) and DPPB (1,4-bis(diphenylphosphino)butane, sold under the reference 8310 by the company Lancaster) in a 1:1 ratio with 2 equivalents of 2-merceptobenzoic

acid (Aldrich, T3,320-0) in CH_2Cl_2 at a concentration of 0.1 molar with respect to product **3** at room temperature for 2 hours. After evaporation of CH_2Cl_2, the reaction mixture is taken up in diethyl ether, and then compound **4b** is obtained in the hydrochloride form by precipitation, by bubbling gaseous hydrochloric acid in solution.

The solvents are purified according to the customary methods in organic synthesis (Purification of Laboratory Chemicals, 2nd edition, D.D. Perrin, W.L.F. Armarego, D.R. Perrin, Pergamon Press, Oxford).

The characterization of these intermediates by conventional methods of Nuclear Magnetic Resonance (NMR; Bruker Spectrospin, Bremen, Germany) and mass spectrometry (MS; MALDI TOF, Protein TOF, Bruker Spectroscopin, Bremen, Germany) was carried out and the data are in agreement with the expected theoretical values.

Description of products **4a** and **4b**:

4a. White solid. ^1H NMR (200 MHz, $CDCl_3$) d(ppm) 1.21 (d, 3H, J=6.6 Hz), 1.4-1.44 (m, 6H), 1.42 (s, 9H), 1.77 (m, 1H), 2.30 (m, 1H), 2.88-3.09 (m, 2H), 3.27 (m, 1H), 3.74 (m, 1H), 4.7 (d, 1H) MALDI-TOF MS: m/z 231.2 ($M+H^+$).

4b. White solid (chloride salt). ^1H NMR (200 MHz, $CDCl_3$) d(ppm) 1.24 (d, 3H, J=6.1 Hz), 1.39-1.47 (dd, 6H, J=6.5 Hz), 1.65-2.08 (2m, 2H), 2.88-3.08 (2m, 2H), 3.26 (m, 1H), 3.71-3.92 (m, 1H), 4.20 (m, 1H), 4.39 (m, 2H), 5.23 (bb, 1H), 7.27-7.41 (m, 4H), 7.59 (d, 2H, J=6.9 Hz), 7.60 (d, 2H, J=6.8 Hz); MALDI-TOF MS: m/z 353.4 ($M+H^+$).

Example 2: Synthesis of a pseudopeptide comprising the carbaza unit B according to formula I.

The synthesis of the peptide is carried out as far as the tyrosine residue from an MBHA resin (100 micromoles of resin with a degree of substitution of 0.63 milliequiv./g, reference 400373 from the company Applied Biosystems) on an Applied Biosystems apparatus (model 431) according to conventional methods of peptide synthesis using the Boc or Fmoc strategy for the protection of the amino acids; (see, for example, Synthetic peptides, a user's guide, published by Gregory A. Grant, WH Freeman and Company, New York, 1992 or The Practice of Peptide Synthesis, published by M. Bodanszky and A. Bodanszky, Springer Verlag, Berlin, 1984). The protected diamino molecule 4a or 4b according to the trials is then coupled to an amine via a carbonylating agent according to the scheme described in figure 2.

The various steps are described below.

1. 10 equivalents of DIEA relative to the initial grafting of the resin (N,N-diisopropylethylamine, Aldrich, D12,580-6) in 2.5 ml of CH_2Cl_2 for 10 min at room temperature.

2. 3.3 equivalents of triphosgene mixed with 10 equivalents of DIEA in 2.5 ml of CH_2Cl_2 for 20 min at room temperature. Other conditions according to the carbonylating agent and the protecting group GP are given in the table below.

3. 5 equivalents of compound **4a** or **4b** in 2.5 ml of CH_2Cl_2 for 1 hour at room temperature.

4. Deprotection of GP.

5. Peptide elongation and final cleavage.

Tyr represents tyrosine, Asn asparagine, Phe phenylalanine, Ala alanine, Thr threonine and Nle norleucine.

The final cleavage with simultaneous deprotection is carried out by mixing strong acids according to the procedure described by Fujii et al. (Fujii, N.; Otaka, A.; Ikemura, O.; Akaji, K.; Funakoshi, S.; Hayashi, Y.; Kuroda Y.; Yajima, H. 1987 J. Chem. Soc. Chem. Commun. 274-275) or with hydrofluoric acid in the case of a synthesis with Boc strategy. With Fmoc strategy, the cleavage is carried out with the K reagent (King, D.; Fileds, C.; Fileds, G. 1990 Int. J. Pept. Protein Res. 36, 255-266). The G. 1990 Into. J. Pept. Protein Res. 36, 255-256). [sic] The cleavage of GP is carried out according to the customary methods in peptide synthesis.

After purification by reversed phase preparative HPLC, the pseudopeptide obtained was characterized by analytical HPLC and mass spectrometry as described in the Limal et al. publication (Limal, D.; Briand, J.P.; Dalbon, P.; Jolivet, M.; 1998, J. Peptide Res. 52, 121-129).

The table below shows the various possibilities of synthesis as well as the coupling yields obtained:

Table

Synthesis strategy: nature of GP	Carbonylating reagent Step (2)	Reaction time for the diamine (hour)	Total yield of synthesis of the pseudopeptide after HPLC purification (%)
Boc	Carbo-diimidazole	1	6

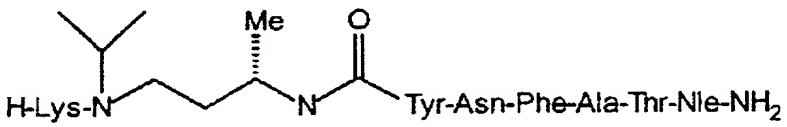
Boc	Carbo-diimidazole	12	30
Boc	Carbo-diimidazole	72	35
Boc	Triphosgene	1	35
Boc	Triphosgene	12	32
Boc	Triphosgene	1	40
Fmoc	Triphosgene	12	25

Retention time for the pseudopeptide by HPLC: 11 min 88 sec.

MALDI-TOF MS: m/z 1012.05 ($M+H^+$) in agreement with the theoretical weight.

Example 3: Synthesis of a pseudopeptide comprising the carbaza unit B according to formula II.

The key molecule for the synthesis of the pseudopeptide is a monoprotected diamine and it is therefore natural to be able to introduce it into the synthesis through either of its amine functional groups. For that, the end comprising the secondary amine of compound 4a was again protected with an Fmoc group and the protected end deprotected with the Boc group. The molecule obtained was introduced onto a solid support in the same manner as above. The molecule represented below is thus obtained with a mass identical to the preceding pseudopeptide compound but with a different retention time.

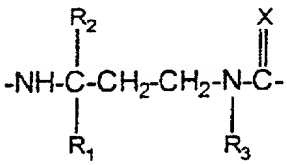


Retention time by HPLC: 11 min 55 sec (HPLC conditions described in example 2).

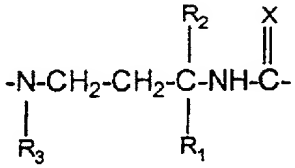
MALDI-TOF MS: m/z 1012.05 ($M+H^+$).

CLAIMS

1. A pseudopeptide of at least 6 amino acids comprising at least one unit chosen from the B units of general formulae (I) and/or (II):



(I)



(II)

in which:

R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical or different,

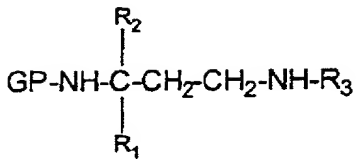
X represents an oxygen or sulfur atom.

2. The pseudopeptide as claimed in claim 1 having a size of at least 9 amino acids.

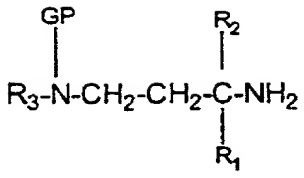
3. The pseudopeptide as claimed in claim 1 or 2, characterized in that X represents an oxygen atom.

4. The pseudopeptide as claimed in any one of claims 1 to 3, characterized in that R_2 represents a hydrogen atom.

5. A method for synthesizing a pseudopeptide as claimed in any one of claims 1 to 4, characterized in that there is used a monoprotected diamine of general formula IIIa or IIIb



(IIIa)



(IIIb)

in which:

R_1, R_2 and R_3 each independently of one another represent an amino acids side chain and may be identical or different,

GP represents a group for protecting the amine functional group.

6. The method as claimed in claim 5, characterized in that GP is a Boc, Fmoc, Cbz or Alloc group.

7. The method as claimed in claim 5 or 6, characterized in that the carbonylating agent is chosen from N,N'-carbonyldiimidazole and triphosgene.

8. A reagent for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, characterized in that it comprises, as reactive substance, at least one pseudopeptide as claimed in any one of claims 1 to 4.

9. The reagent as claimed in claim 8, characterized in that the pseudopeptide is labeled with a tracer or biotin.

10. The reagent as claimed in claims 8 and 9, characterized in that the size of the pseudopeptide is at least 12 amino acids.

11. A kit for detecting a pathological condition associated with the presence of endogenous or exogenous proteins, characterized in that a reagent according to any one of claims 8 to 10 is attached to a solid support which is immunologically compatible with said reagent.

12. A method for detecting and/or assaying biological molecules present in a sample in which the reagent as claimed in any one of claims 8 to 10 is used to form an immune complex with said biological molecules if they are present in the sample.

13. The method of detection as claimed in claim 12, characterized in that the biological molecules are antibodies.

14. A method for detecting and/or assaying an antigen present in a sample by a competition technique in which said sample is brought into contact, simultaneously or in two stages, with a predetermined quantity of an antibody directed against a portion of the antigen and a predetermined quantity of a reagent as claimed in any one of claims 8 to 10, and the presence and/or the quantity of antigen present in said sample is determined.

15. A method for detecting and/or assaying an antibody present in a sample by a competition technique in which said sample is brought into contact simultaneously with a predetermined quantity of an antigen at least a portion of which is recognized by said antibody and a predetermined quantity of a reagent as claimed in one of claims 8 to 10, and the presence and/or the quantity of antibody present in said sample is determined.

16. A monoclonal or polyclonal antibody which can be obtained by immunizing an animal with at least one pseudopeptide as claimed in any one of claims 1 to 4.

17. An anti-idiotype which can be obtained by immunizing an animal with at least one antibody as claimed in claim 16.

18. An active therapeutic composition, in particular an active immunotherapeutic composition, characterized in that it comprises, as active ingredient, at least one pseudopeptide as claimed in any one of claims 1 to 4, an antibody as claimed in claim 16, or an anti-idiotype as claimed in claim 17, said active ingredient being optionally in the form of a conjugate or a pharmaceutically acceptable excipient.

FIG. 1

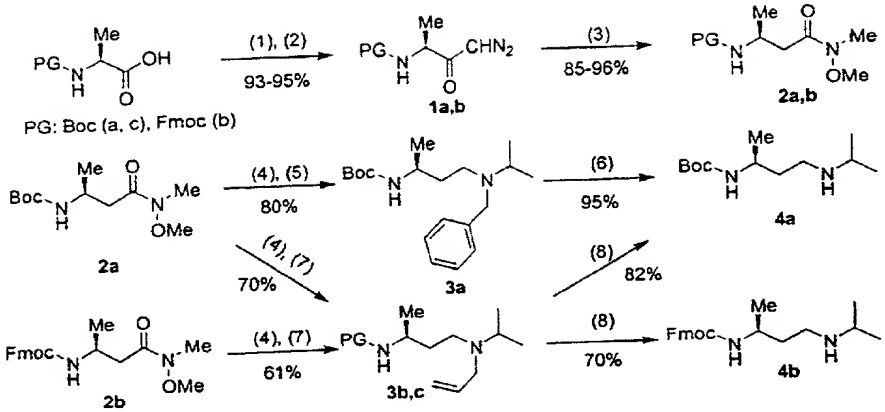
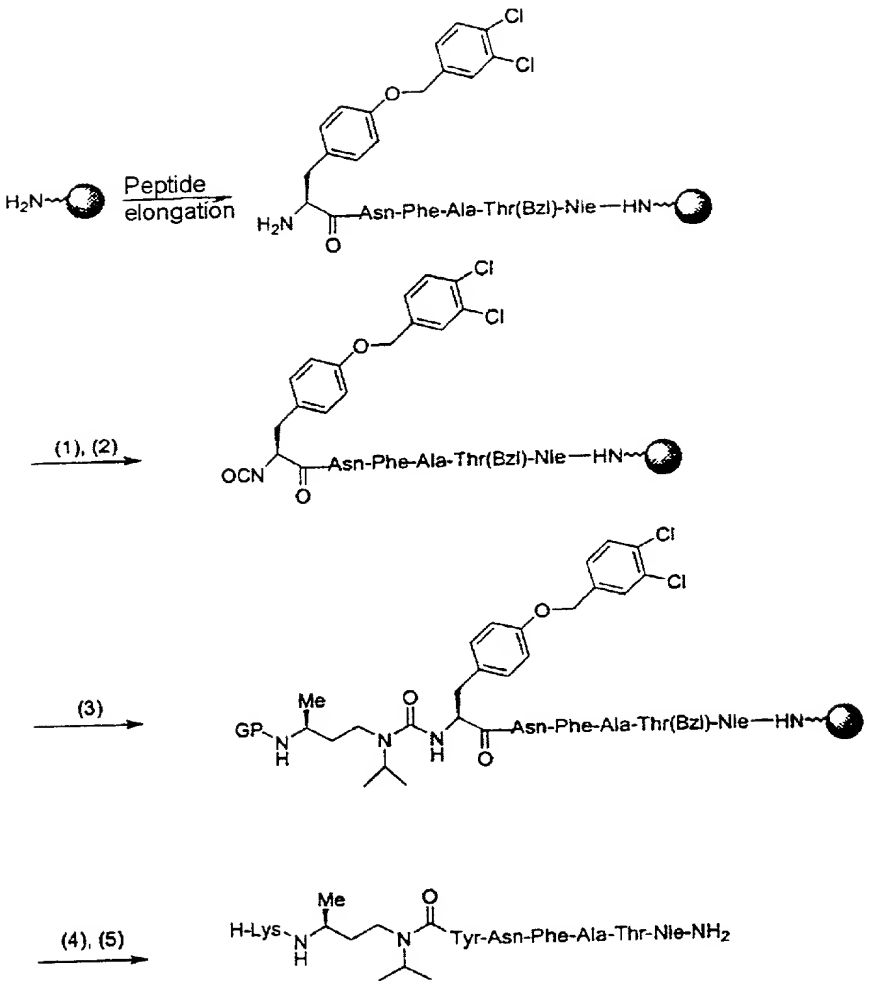

PG: Boc (a, c), Fmoc (b)
(1), (2)
93-95%
(3)
85-96%
1a,b
2a,b
(4), (5)
80%
(6)
95%
4a
2a
(4), (7)
70%
3a
(8)
82%
(4), (7)
61%
(8)
70%
2b
3b,c
4b

FIG. 2


Cl
Cl
O
H_2N
Peptide elongation
H_2N
O
Asn-Phe-Ala-Thr(Bzl)-Nle—HN
(1), (2)
OCN
O
Asn-Phe-Ala-Thr(Bzl)-Nle—HN
(3)
GP
N
H
Me
O
N
N
H
O
Asn-Phe-Ala-Thr(Bzl)-Nle—HN
(4), (5)
H-Lys
N
H
Me
O
N
Tyr-Asn-Phe-Ala-Thr-Nle-NH_2

Docket No.: 110072

DECLARATION AND POWER OF ATTORNEY UNDER 35 USC §371(c)(4) FOR PCT APPLICATION FOR UNITED STATES PATENT

As a below named inventor, I hereby declare that:
my residence, post office address and citizenship are as stated below under my name;

I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought, namely the invention entitled: Pseudopeptide, synthesis method, reagent and applications

described and claimed in international application number **FR00/00053** filed **January 12, 2000** .

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

Under Title 35, U.S. Code §119, the priority benefits of the following foreign application(s) filed by me or my legal representatives or assigns within one year prior to my international application are hereby claimed:

French patent application N° 99 00597 filed on January 15, 1999

The following application(s) for patent or inventor's certificate on this invention were filed in countries foreign to the United States of America either (a) more than one year prior to my international application, or (b) before the filing date of the above-named foreign priority application(s):

I hereby appoint the following as my attorneys of record with full power of substitution and revocation to prosecute this application and to transact all business in the Patent Office:

James A. Oliff, Reg. No. 27,075; William P. Berridge, Reg. No. 30,024; Kirk M. Hudson, Reg. No. 27,562; Thomas J. Pardini, Reg. No. 30,411; Edward P. Walker, Reg. No. 31,450; Robert A. Miller, Reg. No. 32,771; Mario A. Costantino, Reg. No. 33,565; Caroline D. Dennison, Reg. No. 34,494; and Stephen J. Roe, Reg. No. 34,463.

9

ALL CORRESPONDENCE IN CONNECTION WITH THIS APPLICATION SHOULD BE SENT TO OLIFF & BERRIDGE, PLC, P.O. BOX 19928, ALEXANDRIA, VIRGINIA 22320, TELEPHONE (703) 836-6400.

I hereby declare that I have reviewed and understand the contents of this Declaration, and that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1 ***Typewritten Full Name of Sole or First Inventor*** — Given Name: Jean-Paul; Middle Initial: ; Family Name: BRIAND

2 **Inventor's Signature** Jean-Paul Briand

3 **Date of Signature** Month: 12; Day: 17; Year: 2001

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Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

IF THERE IS MORE THAN ONE INVENTOR USE PAGE 2 AND PLACE AN "X" HERE ☒

(Discard this page in a sole inventor application)

1 *Typewritten Full Name of Joint Inventor*

Vincent (Given Name) | Middle Initial | SEMETEY (Family Name)

2 **Inventor's Signature:** Vincent SEMETEY

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1 *Typewritten Full Name of Joint Inventor*

David (Given Name) | Middle Initial | LIMAL (Family Name)

2 **Inventor's Signature:** David Limal

3 **Date of Signature:** 12 (Month) 17 (Day) 2001 (Year)

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1 *Typewritten Full Name of Joint Inventor*

Given Name | Middle Initial | Family Name

2 **Inventor's Signature:**

3 **Date of Signature:** Month | Day | Year

Residence: City | State or Province | Country

Citizenship:

Post Office Address: (Insert complete mailing address, including country)

1 *Typewritten Full Name of Joint Inventor*

Given Name | Middle Initial | Family Name

2 **Inventor's Signature:**

3 **Date of Signature:** Month | Day | Year

Residence: City | State or Province | Country

Citizenship:

Post Office Address: (Insert complete mailing address, including country)

Note to Inventor: Please sign name on line 2 exactly as it appears in line 1 and insert the actual date of signing on line 3.

This form may be executed only when attached to the first page of the Declaration and Power of Attorney of the application to which it pertains.